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BIOMETRIC AND TOTAL CHLOROPHYLL CONTENT ANALYSIS OF MICROGREENS

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Abstract: Microgreens are young seedlings of vegetables and herbs, having two fully developed cotyledons with the first pair of true leaves emerging or partially expanded. Seeds of beetroot, mustard, cabbage, radish and wheat were raised to microgreens by growing under four treatments such as red soil (control), cow dung, cattle waste and NPK. Radish seeds exhibited first pair of leaves on 7^{th} day in all the treatments with the highest germination rate of 83.76% in cow dung treatment. Beetroot seeds exhibited poor germination rate in all treatments compared to other microgreens. Beetroot microgreens showed the development of first pair of leaves on 13^{th} to 15^{th} day in all treatments. Wheat microgreens recorded the highest leaf area of 52.99cm^2 in NPK treatment. Leaf area of beetroot microgreens were very small in all treatments compared to other microgreens. Wheat microgreens grown in cattle waste treatment recorded highest total chlorophyll content of 2.52 µg/g. Leaf area and total chlorophyll content of wheat microgreens were higher than all other microgreens.

Keywords: Microgreens, germination rate, leaf area, total chlorophyll content

1. Introduction

Microgreens are the emerging class of fresh produce and they are tiny greens which is used as the decoration component in fine dining for its attractive flavours. Microgreens are smaller than baby greens and larger than sprouts. It is about 3cm to 6cm long with delicate stem and pair of either fully or partially expanded cotyledon leaves and another pair of young leaves. In recent times, interest towards fresh and functional foods is developing rapidly as the health benefits of microgreens attracted the consumers to develop growing interest.

Growing of microgreens is versatile that it can be grown well in both soil and soil less hydroponic system. Growing conditions directly affect the plant growth and the levels of phytonutrients and minerals. Microgreens can be consumed in various ways such as salad toppings or soup and it is highly utilised by chefs for its attractive flavours.

Microgreens are used as culinary ingredients to enhance the flavours and texture of food and it can be served as sandwiches, soups or can be used as toppings to salads (Pinto et al., 2015)

Microgreens generally have very short shelf-life and thus it requires to be consumed immediately after harvest. Researches are being performed to optimize packages that provides suitable atmospheric composition to extend the shelf life of microgreens after harvest. Plastic clamshell containers which are used for storing microgreens commercially, do not provide the right balance of oxygen and carbon dioxide

Microgreens are harvested by cutting the seedlings manually or mechanically above the ground surface by carefully excluding the growing media particles and seed integuments attached to the cotyledons (Di Gioia et al., 2015). Since the science of is still incomplete, additional research is suggested by the researchers determine the nutrient bioavailability, optimum growing conditions and post harvest stability of nutrients. Microgreens are more nutritious than its mature counterparts and the level of phytonutrients are correlated with the number of days of growth, physiological stage and the rate of photosynthesis. Nutritional value of microgreens makes it an inevitable component in pharmaceutical industry

From wide range of crop seeds, both sprouts and microgreens can be grown in home, either by growing in containers, terrace or commercially. Growing period of sprouts is 3-7 days whereas for microgreens, it is 7-14 days. Growing of microgreens is very economical that it requires no fertilizers and pesticides till harvest. Seeds should be kept in moist condition to facilitate complete germination. During seedling emergence, seeds are covered with clear plastic cover which provides a mini-greenhouse effect. After germination, the seedlings are grown under light with normal humidity and oxygen levels to prevent bacterial contamination. Harvesting in morning is the best time to collect tender sprouts and microgreens. Harvesting only the amount required for the daily meal eliminates vitamin loss which usually occurs during storage. Fresh harvest can also be stored in refrigerator upto one week. Microgreens which are usually larger than sproutsand smaller than baby greens, have superior range of flavor aroma, textures and colours. These qualities are attributable to different growing conditions. (Treadwell, 2010).

Chlorophyll and carotenoids have potential role in treating thalassemia, hemolytic anemia and reduce the risk of some chronic diseases such as cancer by limiting the bioavailability of carcinogens, cardiovascular diseases, skin diseases and age-related eye diseases. Chlorophyll is found to be effective in treating many forms of infections and heals wounds and inflammations (Niroula et al.,2019). Germination process increases the nutrient levels such as vitamins, antioxidants and reduce the levels of antinutrients such as phytic acids (Seneviratne et al., 2019). Level of phytoconstituents differ according to growth stages of the plant and it often decrease from the seedling to the fully developed stage (Ebert et al., 2015). Microgreens are higher in nutritional value than mature vegetables and the amount of chlorophyll, carotenoids, vitamins and enzymes varies with crop, method of growing, media used and time of harvesting the microgreen. Highly nutritional microgreens are intensely coloured than lighter ones (Choe, 2018).

Within 6 days, radish exceeded the maximum height limit of 6 cm and on day 8, true leaves emerged. Microgreens of lettuce, mustard and sesame exhibit moderate rates of growth and did not exceed the maximum height within 14 days (Gayathree et al, 2019). Beet microgreens are rich source of minerals, carotenoids, flavonoids and vitamins which are helpful in protecting from contracting lung or mouth cancer (Rickman, 2007). Radish microgreens contains various nutrients such as carbohydrates, vitamins, proteins and minerals which serves in the prevention of human health issues (Xiao, 2012). Brassica microgreens are found to contain 165 phenolic compounds comprising many highly glycosylated and acylated quercetin, kaempferol, cyanadin aglycones, and complex hydroxycinnamic and benzoic acids than in their mature plant counterparts (Sun et al., 2013)

Huang et al. (2016) found that supplementation with cabbage microgreens is good for weight gain and decreases the level of low density lipoproteins (LDL), hepatic cholesterol esters and triglyceride levels and inflammatory cytokines.

Cultivation of microgreens is possible indoor, outdoor and controlled environmental conditions, depending on the scale of production. After each harvest, microgreens should be stored in containers to retain the quality of greens in terms of freshness and flavour. This method prevents the harvest and many postharvest handling issues (Bhatt and Sharma, 2013). Consumption and utilization of microgreens has been increased due to the high levels of functional compound which increased public interest towards health promoting lifestyle. Microgreens are grown from variety of crops such as radish, lettuce, mustard, cabbage, broccoli, amaranth (Burlingame. 2014).

2. Objectives of the study

Goal of this work was to examine the following

- to understand the ease of growing microgreens
- to know about growing microgreens in reduced time and cost of production
- to assess and examine the biometric observations such as shoot length, yield and germination percentage
- to analyse the total chlorophyll content of each microgreens

3. Materials and methods

3.1 Procurement of seeds

Seeds of beetroot, cabbage, radish, mustard and wheat were obtained from the commercial seed sellers. Seeds were washed well with tap water to remove surface pollutants

3.2 Experimental methodology

Microgreens were grown in red soil in four different treatments which includes cow dung, cattle waste, NPK fertilizer and control (Red soil).

3.2.1 Microgreen production

Seedlings were raised in plastic trays in triplicates. Trays were filled with respective treatments and seeds were sown in it. Dried cattle waste and cow dung were powdered coarsely and then utilized for plant growth. Growing media was kept moist or wet by spraying water 2-3 times a day. Microgreens were allowed to grow for 7-14 days.

3.2.2 Harvesting microgreens

Microgreens were harvested after the expansion of two true leaves. Harvest period differs for each microgreens. At the time of harvest, microgreens were certain inches tall than sprouts. Plants were cut at the soil surface and the roots were left untouched.

BEETROOT

MUSTARD

CABBAGE **RADISH** **WHEAT**

Figure 1. Growing of microgreens

3.3 Observations recorded

3.3.1 Biometric observations

Germination percentage

Germination rate of seeds were calculated by determining the number of seeds germinated and total numberof seeds sown. By this method the germination % was calculated.

$$\text{Germination rate} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Leaf area of microgreens

Leaf area of microgreens were measured using scale at the time of harvest for each replications and mean length was calculated and expressed in centimeter square. Leaf area was determined by the length and width of the leaves

Leaf area = Leaf length × Leaf width

Length of leaves was determined by measuring the leaf along the vein and width was determined by measuring the width of central portion of the leaf lamina.

3.3.2 Biochemical evaluation

Chlorophyll- a, b and total chlorophyll (Arnon, 1945)

Amount of chlorophyll-a, b, and total chlorophyll was determined by Arnon (1945) method with slight modifications using spectrophotometer and their mean amount was expressed in µg /g.

Procedure:

0.5 g of each leaf sample was weighed and macerated with 10 ml of 80% acetone. It was then centrifuged at 3000 rpm for 10 minutes. Supernatant was collected and the volume wsa made upto 25 ml by using 80% acetone. OD values were measured at 645nm and 663nm in spectrophotometer.

Calculation

The chlorophyll content of the samples was expressed in µg /g of fresh leaf.

$$\text{Chlorophyll a} = \frac{(12.7 \times \text{OD at } 663) - (2.69 \times \text{OD at } 645) \times v}{1000 \times w}$$

$$\text{Chlorophyll b} = \frac{(22.9 \times \text{OD at } 645) - (4.68 \times \text{OD at } 663) \times v}{1000 \times w}$$

$$\text{Total chlorophyll} = \frac{(20.2 \times A\ 645\text{nm}) + (8.02 \times A\ 663\text{nm})}{1000 \times w} \times v$$

Where

A = Absorbance at specific wavelength (nm)
V = final volume of supernatant (ml)
W = weight of leaf sample (g)

4. Results and discussion

5.1 Biometric parameters

Radish

Radish seeds exhibited highest germination rate in all treatments than other microgreens. Maximum germination rate of radish was found in cowdung treatment, which is 83.76%. It exhibited faster growth and first pair of true leaves emerged on 7 th day in all treatments. Leaf area of radish was found highest in NPK treatment (5.66cm).

Soil containing cow dung exhibits maximum seedling height as cow dung possess optimal water holding capacity and aeration which facilitates imbibition, hydration of protoplasm and restoration of enzymatic activity, favouring entire germination processes (Polash et al., 2019)

Wheat

Seeds of wheat microgreens exhibited second highest germination rate in all treatments than all other microgreens. It was found highest in cow dung treatment (82.05%). First pair of true leaves emerged on 8th to 10th day in all treatments. Wheat microgreens showed highest leaf area in all treatments than the rest of the microgreens. Wheat greens grown in NPK treatment possessed maximum leaf area of 52.99cm

Cabbage

Maximum germination rate of cabbage was found in control, which is 84.6%. First pair of true leaves emerged on 9th to 10th day in all treatments. In NPK treatment, leaf area of cabbage microgreens was to found to be 5.33cm which was highest than those found in other treatments.

Mustard

Mustard exhibited good germination rate in all treatments. However, germination percentage of mustard is highest in Cow dung treatment, which was found to be 67.52%. First pair of true leaves emerged on 10^{th} to 11^{th} day in all treatments. Mustard microgreens grown in NPK treatment possessed maximum leaf area of 6cm

Beetroot

Beetroot seeds exhibited poor germination rate in all treatments compared to other microgreens. Beetroot seeds has shown maximum germination rate of 20.86% in cow dung treatment. First pair of true leaves emerged on 13^{th} to 15^{th} day in all treatments. Leaf area of beetroot was very small in all treatments compared to all other microgreens. However leaf area of beetroot was found highest in NPK treatment (4.33cm)

Table 1. Leaf area and Germination percentage of microgreens

Plants	Control (T1)		Cow dung (T2)		Cattle waste (3)		NPK (T4)	
	Leaf area(cm^2)	Germination %	Leaf area(cm^2)	Germination %	Leaf area(cm^2)	Germination %	Leaf area(cm^2)	Germination %
Beetroot	2.16±0.2	7.69±0.5	2.86±0.89	20.51±0.96	3.14±0.6	20.51±0.72	4.33±0.43	7.69±0.59
Mustard	3.76±0.23	56.4±0.35	3.21±0.25	67.52±0.92	3.13±0.12	56.41±0.23	6±0.48	47.8±0.23
Cabbage	3.9±0.21	84.6±0.67	4.45±0.28	82.9±0.56	3.37±0.19	71.79±0.65	5.33±0.15	35.89±0.69
Radish	2.980.13±	81.19±0.83	4.6±0.6	83.76±0.85	3.66±0.56	77.77±0.76	5.66±0.23	54.7±0.69
Wheat	33.66±0.55	72.64±0.62	44±0.39	82.05±0.59	48±0.68	76.06±0.69	52.99±0.51	73±0.71

***Mean ± Standard deviation**

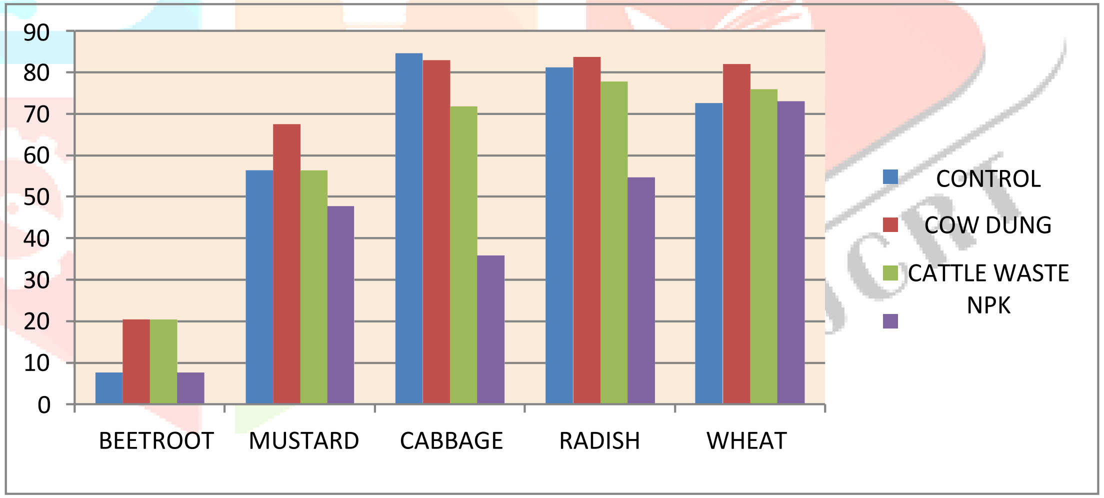

Figure 2. Germination percentage of microgreens

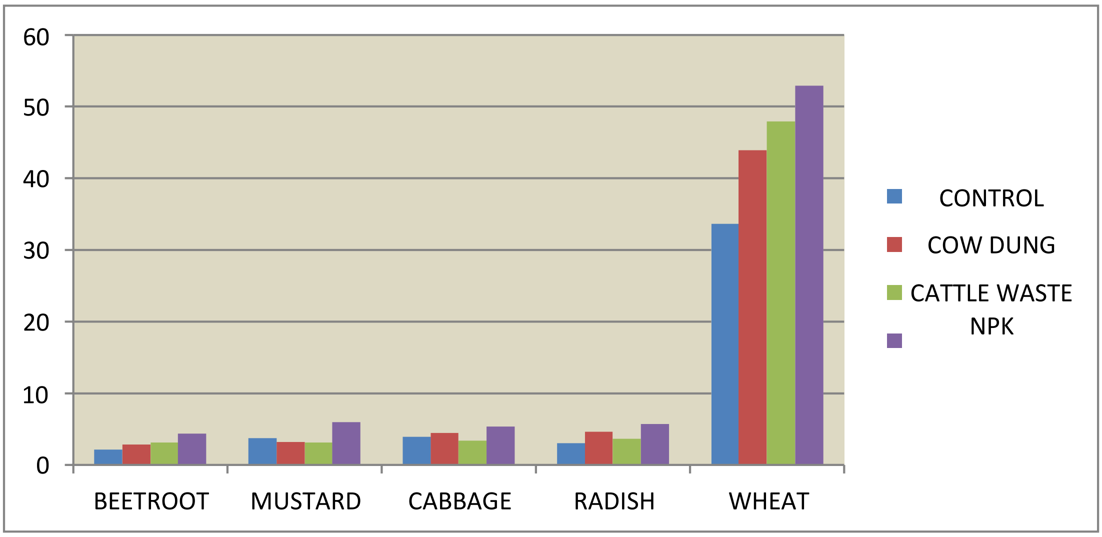

Figure 3. Leaf area of microgreens

5.2 Total chlorophyll content of microgreens

Total chlorophyll content was highest in wheat microgreens in all treatments compared to other microgreens. Wheat microgreens grown in cattle waste treatment recorded high total chlorophyll content of about 2.52 µg/g. Radish exhibited relatively level of total chlorophyll content in all treatments. Total chlorophyll content of radish grown in cow dung treatment was found to be highest (1.01 µg/g). In control (red soil), cabbage microgreens recorded highest total chlorophyll content of 1.67 µg/g. Mustard microgreens grown in cow dung treatment recorded high total chlorophyll content of about 1.07 µg/g. Total chlorophyll content of beetroot grown in cattle waste treatment was found to be highest (1.17 µg/g).

Table 2. Total chlorophyll content

	Control (T1) (µg/g)	Cow dung (T2) (µg/g)	Cattle waste (T3) (µg/g)	NPK (T4) (µg/g)
Beetroot	0.42±0.32	0.46±0.36	0.01±0.31	0.11±0.11
Mustard	0.31±0.28	0.33±0.37	0.24±0.35	0.20±0.19
Cabbage	0.35±0.54	0.52±0.36	0.42±0.23	0.49±0.31
Radish	0.01±0.57	0.38±0.33	0.13±0.1	0.37±0.18
Wheat	0.50±0.47	0.71±0.39	0.70±0.91	0.36±0.97

***Mean ± Standard deviation**

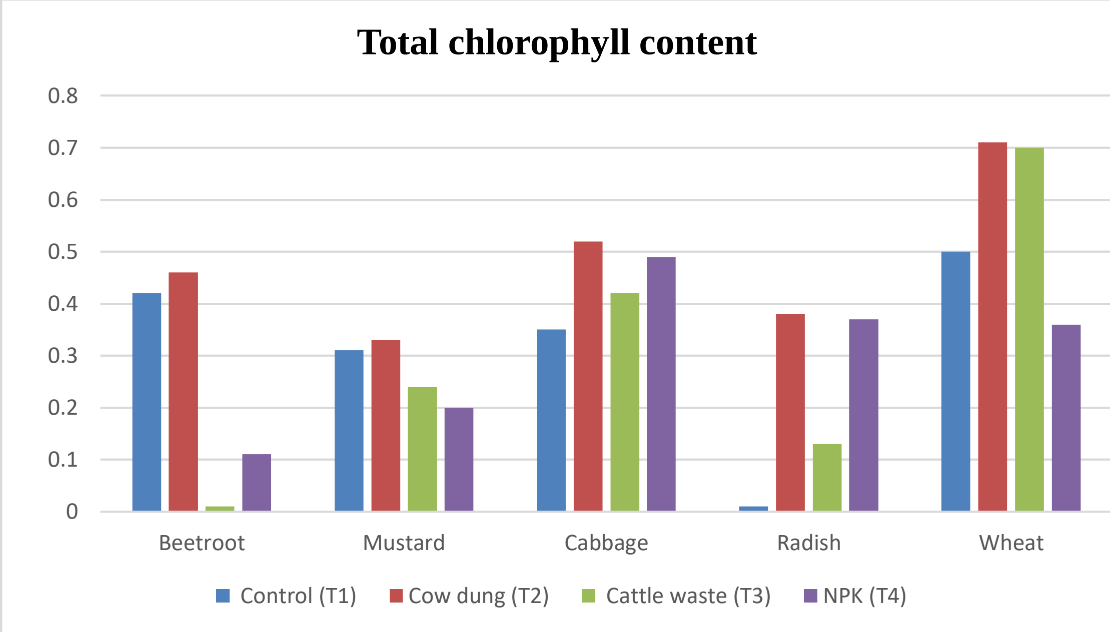

Figure 4 . Total chlorophyll content

Conclusion

Microgreens are the first true leaves produced from a seedling of vegetables or herbs that are about 2-3 inches tall. This study revealed that microgreens are easy to grow in very short duration. It can be grown in a limited space, thereby contributing an advantage to urban growers. It can be harvested within 10-20 days after germination and pays us premium income due to its demand in hotels and restaurants. Radish microgreens exhibited faster development within 7 days in all treatments than other microgreens. Beetroot microgreens exhibited poor germination rate and possess minimum chlorophyll content. All the five microgreens possess highest germination rate and total chlorophyll content in cow dung treatment. This was due to the nutrients contained in cow dung and the organic matters present in cow dung provides good water holding capacity and aeration to the soil. Leaf area of wheat is larger than all microgreens and ultimately total chlorophyll content was also found to be present in higher quantity.

As people are becoming directed towards healthy practice due to the fear on emerging diseases, this work will grab their attention. Microgreens will meet their nutrient adequacy, enhance their interest towards agriculture and will contribute to a good source of profitable business.

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